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(54) Title: ANTISENSE TREATMENT OF PULMONARY HYPERTENSION		
(57) Abstract		
<p>The invention herein described relates to a method to treat pulmonary hypertension by antisense therapy using ET-1 derived antisense molecules delivered to the lungs as a pulse/spike in an inhaler.</p>		

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ANTISENSE TREATMENT OF PULMONARY HYPERTENSION

The invention relates to a method and means to treat, but not exclusively, pulmonary hypertension by functionally suppressing the product of the ET-1 gene which encodes a vasoconstrictor peptide the expression of which is
5 correlated mainly with this disorder.

Pulmonary Hypertension (PH) is a disorder in which the blood pressure in the pulmonary (lung) arteries is abnormally high in the absence of other diseases of the heart or lungs. PH is considered to be present when the mean pulmonary arterial pressure is greater than 25mmHg at rest or 30mmHg
10 during activity. The normal mean pulmonary – artery pressure is approximately 14mmHg at rest. PH is classified according to whether the disorder is primary or secondary PH.

Primary PH (PPH) is a relatively rare disorder affecting mainly women between the ages of 21 and 40 although men and children can suffer from the
15 disease. The incidence annual rate is as high as 2 per million of the UK population. In the USA approximately 300 new cases of PPH are diagnosed each year. The exact cause of PPH is unknown, this is mainly due to the low incidence of PPH and the lack of an effective animal model in which to study the disease. However PPH is associated with cirrhosis, AIDS and can
20 be familial.

Secondary Pulmonary Hypertension (SPH) is present where there is an identifiable cause resulting in an increase in pulmonary arterial pressure. These include left ventricular failure, chronic hypoxia, lung disease or alveolar hypoventilation and a number of systemic diseases such as lupus

erythromatosis, systemic sclerosis, SPH may also result from chronic thrombo-anabolic disease.

For both PPH and SPH the symptoms are similar. These include breathlessness, syncope (faints), angina (chest pain), headaches, fatigue, blurred vision, insomnia, and intestinal complaints. SPH is much more common and is a major killer in the Western world.

There are currently a number of drug treatments that can help to ameliorate pulmonary hypertension. The successful clinical treatments include the continuous intravenous administration of prostacyclin (PGI_2) and inhaled nitric oxide (NO), for severe disease; for milder degrees of illness, anticoagulants and calcium channel blockers can be used to lessen symptoms and improve survival.

In pulmonary hypertension, sufferers respond differently to different drugs therefore no single drug regime can be used to blanket treat sufferers. Moreover dosages have to be empirically decided due to the potential for adverse side effects. For example, systemic low blood pressure, nausea, angina, headaches and flushing. Therefore alternative strategies are required to provide therapeutic treatment of pulmonary hypertension.

The product of the gene endothelin-1 (ET-1) has been shown to act as a potent vasoconstrictor and is involved in the remodelling of blood vessels of the lung to cause narrowing and obstruction. The product of the ET-1 gene is found in endothelial cells that line arteries and has been shown to be elevated in expression in individuals and in animal models that show pulmonary hypertension. Moreover, current successful treatments of clinical and experimental hypertension using PGI_2 , and NO have been shown to

inhibit the expression of ET-1. This correlative data is strongly suggestive that ET-1 is an important component in mediating pulmonary hypertension.

Indeed, the drug BQ-123 has been shown to antagonise the activity of ET-1 receptors and relieve pulmonary hypertension resulting from congestive heart failure. In rat models, mRNA to ET-1 was shown to decrease as a
5 consequence of long term BQ-123 treatment.

The problems of drug side effects, amongst other things, has lead to alternative therapeutic strategies to be devised. A considerable effort is being made toward developing gene therapy approaches to control disease
10 pathogenesis and it is likely that in subsequent years this will provide a serious alternative to the use of pharmaceuticals.

A recent strategy to interfere with the expression of a gene is that of antisense technology. In brief this strategy involves the use of a DNA or RNA molecule that is complementary to a region of a selected gene and is
15 able to hybridise (bind) under physiological conditions to the targeted nucleic acid to prevent either transcription of the gene or translation of the mRNA encoded by the gene. The antisense molecule is often a short oligodeoxynucleotide (ODN). However the molecule may be an oligodeoxyribonucleotide, or a modified oligodeoxynucleotide, or a
20 modified oligodeoxyribonucleotide; each of which are able to hybridised to a selected part of a gene, or mRNA, under physiological conditions. The modifications to oligodeoxynucleotides will be apparent to one skilled in the art. The exact region of the nucleotide sequence of the gene to which the antisense molecule is designed can be empirically determined. However it is
25 common practice to design oligodeoxynucleotides to the 5' region of the gene (to interfere with transcription initiation) or the mRNA (to interfere

with translation). As stated before, the region of the gene to which the antisense molecule is directed is determined by the efficiency with which the antisense molecule suppresses the gene of interest. Contrary to the above this may be the 3' region and is determined experimentally. The length of the ODN also has to be determined experimentally. Typically ODNs are 20-30 nucleotides in length but may be much longer.

Presently, a number of delivery methods are being explored for their effectiveness at delivering gene therapy to a specific tissue. Viral vectors and liposomes have been used with some success. Recently, cationic lipids chelated to a therapeutic DNA molecule have been shown to deliver the DNA molecule to the lungs (1). Cationic lipids are positively charged lipids which when combined with negatively charged DNA form a tight complex. Moreover, the lungs are coated in a surfactant, a major component of which is dipalmitoylphosphatidylcholine, which is cationic at physiological pH and which is internalised by endothelial cells that line the arterioles of the lungs.

In our co-pending application (GB9805185.7 ref. 2) the delivery of an aerosol containing a vasodilator by a specialised inhaler is described. As an alternative to the continuous perfusion of the lungs using a nebuliser, the therapeutic composition, ie aerosols, is delivered intermittently and in short pulses using the so called "spike principle". By using this methodology the medicament is driven deep into the lung tissue only during inhalation. There are many lung diseases and conditions suffered by humans for which the preferred form of treatment involves delivering a medicament of the appropriate sort down a patient's airways into the lungs, where the medicament can act upon, and perhaps be absorbed into, the tissues of the lungs. The most effective treatments for these disorders involve the inhalation of some suitable chemical agent in inhaled air. The co-pending

application describes the administration of nitric oxide, a known vasodilator, in the form of a pulse or "spike". In the treatment of the constriction of the small pulmonary arteries, the very short pulse of nitric oxide is provided at the start of the inhalation, such that the resultant bolus of nitric oxide mixture
5 inhaled by the patient has a nitric oxide concentration high enough to have the desired therapeutic effect, even if admixed with some additional air, but is of such short duration (both in time and as a result, in physical amount) that, pushed by the following much larger volume of plain, and therefore nitric oxide – free, air/oxygen, it reaches deeper into the lungs, where it both
10 acts on the small pulmonary arteries and is taken up into the capillaries,

The invention therein described is a small (pocket-sized) hand held treatment apparatus that utilises the "spike" principle. The invention comprises a conventional nebuliser (having a reservoir in which is stored the medicament to be administered) driven by some suitable pressurised gas from a valved
15 cylinder so as to deliver a medicament into a tube through which the patient is breathing (by mouth) normal air, the gas cylinder valve being controlled by a suitably programmed computing device that is fed data describing the pressure within the breathing tube and so is able to open and close the valve at, and for, a time such as to drive the nebuliser to deliver (to the tube and
20 thence to the patient's lungs) a required pulse of medicament at any selected point within the patient's respiratory cycle. The idea of this is simply to ensure that a high concentration of the medicament reaches and affects the target area, and the target area alone, rather than having the whole of the lungs subjected to it.

25 The apparatus of the invention incorporates a nebuliser in the reservoir of which is storable the medicament to be administered. The reservoir has a fine orifice through which its contents may exit and across which is blown a

stream of gas along a pipe way. As the gas flows past the orifice it causes an external pressure drop (relative to the reservoir's internal pressure) resulting in some of the reservoir's contents being sucked out into the gas stream creating an aerosol-like cloud of fine drops or particles that is swept along the pipe way by the driving gas. The driving gas is contained within a
5 valved cylinder, the operation of the valve being controlled by a computing means in response to the input from a pressure sensor. The pressure sensor is located within the breathing tube and provides the computing means with data about the air pressure there within, and thus about the patient's
10 respiratory cycle.

The invention therefore comprises a nebuliser with a reservoir containing a therapeutic composition which has contact with a gaseous supply through which the therapeutic composition is aerosolised. The delivery of the composition via the breathing tube is regulated by a valved cylinder which
15 operates according to pressure data received from a pressure sensing means located in the breathing tube to determine the stage of the patient's respiratory cycle. The whole system is computer controlled to ensure that the pulse of therapeutic composition is delivered at the appropriate stage of the patient's respiratory cycle.

20 We have used an inhaler employing the above 'spike' methodology and in one example the specialised inhaler described in GB9805185.7 to create an aerosol of ET-1 antisense molecules to perfuse lung tissue to relieve pulmonary hypertension. The designed antisense ET-1 molecules will target either the ET-1 gene and/or the ET-1 mRNA to suppress the ET-1 gene
25 product from the endothelial cells that line the lung arterioles and thereby relieve pulmonary hypertension.

It is therefore an object of the invention to design antisense ODNs to ET-1 for use in the alleviation of pulmonary hypertension.

It is yet a further object of the invention to deliver antisense ODNs to the lungs to alleviate pulmonary hypertension.

- 5 According to a first aspect of the invention there is provided a method to treat pulmonary hypertension comprising administering a therapeutic composition to an individual, wherein said composition comprises at least one aerosolised antisense ET-1 molecule, delivered to the lungs by an inhaler during inhalation by a patient.

10

Ideally said antisense material is of mammalian origin, and most ideally of human origin.

- It will be apparent from an analysis of the prior art that the biological effects of ET-1 are mediated through the receptors ET-A and ET-B. Therefore
15 although embodiments of the invention will be described that target ET-1 with antisense ET-1 molecules it will be apparent that targeting ET-A and/or ET-B may achieve the desired result of suppressing ET-1 activity although not directly. Therefore the following description assumes that one skilled in the art would appreciate that the antisense strategy could be applied to
20 suppress ET-A and/or ET-B activity.

According to a further aspect of the invention there is provided a method to treat pulmonary hypertension comprising delivering aerosolised antisense ET-1, or ET-A or ET-B molecules to the lungs by an inhaler as a pulse/spike during inhalation.

According to yet a further aspect of the method there is provided a therapeutic composition for treating pulmonary hypertension comprising; the non-coding strand of the ET-1 gene, or fragment thereof, as represented in Figure 1.

5

It will be apparent from Figure 1 that the sequence presented is the sense strand of the ET-1 gene. Antisense molecules would be designed to represent sequence complementary to this sequence and can be easily deduced from the sense sequence.

10 In a preferred method of the invention said antisense ET-1 molecule is a oligodeoxynucleotide, a modified oligodeoxynucleotide, an oligodeoxyribonucleotide or a modified oligodeoxyribonucleotide represented by the DNA sequence shown in Figure 1, that is the non-coding strand of ET-1.

15 Reference herein to modifications is intended to include without limitation modifications apparent to those skilled in the art that improve stability (both as a stored composition and during *in vivo* use; improve permeability across biomembranes or facilitate aerosolisation).

In a preferred method of the invention the antisense molecule is between 10-
20 100 nucleotides in length.

In yet a further preferred method of the invention said antisense molecule is greater than 100 nucleotides in length.

In yet still a further preferred method of the invention said oligonucleotide has a G + C content of at least 50% and more preferably still a G + C content of between 50% and 60%.

- 5 More preferably still said oligonucleotides do not contain a consecutive run of more than four bases of any one kind.

In yet still a further preferred embodiment of the invention said oligonucleotide has a predicted melting temperature of at least 55⁰, and more
10 preferably still, a melting temperature of between 55⁰ – 80⁰.

In a preferred embodiment of the invention there is provided at least one human ET-1 antisense oligonucleotides as represented in Table 1.

15

Table 1

HsET117	CAG CCC AAG TGC CCT TTA AC
HsET132	CTC AAA GCG ATC CTT CAG CC
20 HsET318	AGC TCA GCG CCT AAG ACT GC
HsET438	TGG CAG AAG TAG ACA CAC TC
25 HsET779	TGG TCT CCG ACC TGG TTT GT
HsET868	ATG TGC TCG GTT GTG GGT CA

In yet a further preferred embodiment of the invention there is provided at
30 least one rat ET-1 antisense oligonucleotides as represented in Table 2.

Table 2

RnET198	ACA GCA GAG AGA AGA TCA CG
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	RnET294	TGC ACT TCC TTC TCA GCT CG
	RnET466	GGA TCG CTT AGA CCT AGA AG
5	RnET718	CTT GAT GCT GTT GCT GAT GG
	RnET795	AGT CAA TGT GCT CGG TTG TG
10	RnET1081	ACT GTG TCT CTG CTC TCC GA

It will be evident to one skilled in the art, and from the above description, that a reliable animal model to test the efficacy of therapeutic treatments for pulmonary hypertension currently does not exist. It would therefore be desirable to develop an animal model system to test the efficacy of antisense ET-1 molecules. The sequences identified in table 2 represent preferred rat sequences. However it will be apparent to one skilled in the art that oligonucleotides derived from other species may also be useful in practising this method of the invention. Particularly those sequences that have a high degree of sequence homology to those sequences presented in Tables 1 and/or 2.

According to a further aspect of the invention there is provided a method for determining the efficacy of antisense ET-1 molecules comprising exposing a known hypersensitive animal model system to antisense ET-1 for studying molecules and observing the effects of same on pulmonary hypertension.

In a preferred embodiment of the invention said therapeutic treatment is based on antisense therapy. Ideally said antisense therapy is based on the ET-1 sequence and more preferably still those sequences represented in Table 1 and/or Table 2, and/or sequences homologous or analogous thereto,

wherein said homology is at least 50%, ideally 75% and preferably at least 90%.

In yet a further preferred method of the invention there is provided a modified antisense ET-1 molecule as herein described for the treatment of pulmonary hypertension.

It will be apparent to one skilled in the art that a number of potential
5 modifications can be made to the antisense oligodeoxynucleotide molecules to improve efficacy and/or stability of said molecules. These include modifications to the phosphodiester bonds between nucleotides, (eg the inclusion of peptide bonds to form peptide nucleic acids). Other modifications may be to the bases and/or sugars by the covalent attachment
10 of chemical groups to specific sites in the sugar and/or base. All of these modifications will not affect the binding properties of the antisense molecule to its target site in ET-1.

According to a further aspect of the method there is provided an antisense molecule adapted to hybridise to the transcriptional promoter region of the
15 ET-1 gene to inhibit transcription of said gene.

It will be apparent from an analysis of the prior art that the biological effects of ET-1 are mediated through the receptors ET-A and ET-B. Therefore although embodiments of the invention will be described that target ET-1 with antisense ET-1 molecules it will be apparent that targeting ET-A and/or
20 ET-B may achieve the desired result of suppressing ET-1 activity although not directly. Therefore the following description assumes that one skilled in the art would appreciate that the antisense strategy could be applied to suppress ET-A and/or ET-B activity.

It will be apparent to one skilled in the art that the antisense ET-1 molecule used in this way can be designed to either the sense strand or antisense strand of the ET-1 transcriptional promoter as the target sequence is double stranded genomic DNA.

- 5 According to yet a further aspect of the invention there is provided an antisense molecule adapted to hybridise to splice junctions between exons and introns of prepromessenger RNA encoding the ET-1 protein.

10 It will be apparent to one skilled in the art that the expression of the ET-1 gene can be inhibited by interfering with the splicing of introns from the pre mRNA of ET-1 to prevent the formation of a mature, translatable mRNA.

According to a further aspect of the invention there is provided an inhaler comprising means for administering the aforescribed antisense molecules encoding ET-1 nucleic acid wherein said administration is via a spike or
15 pulse and preferably only during inhalation by the patient.

According to yet a further aspect or embodiment of the invention said inhaler is disposable and adapted to receive a cartridge containing antisense molecules encoding the ET-1 nucleic acid composition.

- 20 According to yet a further aspect of the invention there is provided a method for administering the composition of the invention comprising;

- i) adding or providing the antisense molecular composition in a suitable diluant, carrier or excipient and in dosage form to, or in a reservoir of, an inhaler;

- ii) aerosolising said composition by a suitable means;
- iii) detecting the air pressure within the inhaler by a suitable sensing device to determine a patient's respiratory cycle; and
- iv) delivering a prescribed dose of the afore described therapeutic composition to the patient's lungs during a selected inhalation phase of the patient's respiratory cycle.

In yet a further aspect of the method there is provided a kit used to treat pulmonary hypertension comprising; a therapeutic antisense composition as hereindescribed; an inhaler, including a nebuliser, a reservoir for containment of said composition, an air pressure sensing device and a valve containing pressurised cylinder means to regulate the dosage of said composition.

The invention hereindescribed therefore provides a means of controlling pulmonary hypertension using aerosolised antisense molecules to the ET-1 gene, and/or mRNA, to inhibit the vasoconstrictor activity of the ET-1 peptide. Moreover, the delivery of the antisense molecule makes use of an inhaler that functions during patient inhalation to drive the therapeutic composition deep into the lungs to deliver the antisense molecule to the endothelial cells that line the arterioles of the lungs and thereby target those blood vessels that result in pulmonary hypertension.

An embodiment of the invention will now be described, by example only, and with reference to the following Figure and methods wherein;

Figure 1 represents the genomic DNA sequence of human ET-1.

Antisense Design Protocol

Table 1

5 Human sequences

HsET117	CAG CCC AAG TGC CCT TTA AC
HsET132	CTC AAA GCG ATC CTT CAG CC
HsET318	AGC TCA GCG CCT AAG ACT GC
10 HsET438	TGG CAG AAG TAG ACA CAC TC
HsET454	CCA AAT GAT GTC CAG GTG GC
HsET779	TGG TCT CCG ACC TGG TTT GT
HsET868	ATG TGC TCG GTT GTG GGT CA

15 Table 2

Rat sequences

RnET198	ACA GCA GAG AGA AGA TCA CG
RnET294	TGC ACT TCC TTC TCA GCT CG
RnET466	GGA TCG CTT AGA CCT AGA AG
20 RnET718	CTT GAT GCT GTT GCT GAT GG
RnET795	AGT CAA TGT GCT CGG TTG TG
RnET1081	ACT GTG TCT CTG CTC TCC GA

The above sequences were designed by identifying regions of loose
 25 predicted secondary structure and choosing sequences of 20 base pairs
 meeting the following criteria;

- a) 50-60% G+C content
- b) predicted melting temperature of 55-80 °C

c) <4 runs of bases of any kind

Regions of loose secondary structure were defined as sequences of >8 adjacent bases in the mRNA sequence for which there were no predicted
5 intra-molecule Watson-Crick base pairing determined using the Wisconsin Genetics Computer Group software package RNAFOLD.

The mRNA sequence was then imported into the 'primer' package on the Sheffield 'biocomp service' and complemented (G for C, T for A etc.). The
10 regions of loose predicted secondary structure were sought and the oligo criteria were selected. The 3' most bases were aligned with the region of no predicted secondary structure. If the sequence highlighted met all set criteria the sequence and position was recorded. If the sequence failed to reach the criteria the highlighted 20 base sequence was shifted 5' until either a
15 sequence was identified meeting the criteria or the <5 bases remained within the region of loose predicted secondary structure at which point the region was rejected. When more than one 20 base sequence met the criteria at a particular region of loose predicted secondary structure the sequence with the most central homology to that of the loose predicted secondary structure
20 was selected.

Current data relating to the efficacy of ET-1 antisense material is *in vitro* cell culture work. Selected antisense molecules are transfected into cells in culture and the level of ET-1 protein determined by western blotting and/or
25 immunofluorescence using ET-1 antisera. A down regulation of ET-1 protein results when selected antisense molecules are used. Currently we are unable to identify the mechanism by which ET-1 protein is down regulated (ie destabilisation of mRNA, inhibition of translation). Further work will

optimise conditions with respect to providing a suitable composition for use in *in vivo* studies.

The working of the invention is undertaken by using conventional techniques which will therefore not be described herein in great detail. Suffice to say, that the hereindescribed antisense material is manufactured in conventional manner using laboratory techniques and then suspended in a suitable fluid for the purpose of delivery (3). When thus manufactured the antisense material is suitably contained, ideally in a cartridge, which cartridge is adapted for use in a conventional inhaler and more preferably the inhaler described herein (2).

In this way, the invention is worked in a conventional fashion.

- 15 1 Delivery of genes using cationic lipid US patent no. 5641662
- 2 Inhalers. UK patent application no. 9805185.7.
- 3 Nyce, J.W. and Metzger, W.J. DNA antisense therapy for asthma in
20 an animal model. Nature 285: p721-725. 1997.

CLAIMS

1. A method to treat pulmonary hypertension comprising administering a therapeutic composition to an individual, wherein said composition
5 comprises at least one aerosolised antisense ET-1 molecule, delivered to the lungs by an inhaler during inhalation by a patient.
2. A method according to Claim 1 wherein said composition is delivered to said lungs as a pulse/spike during inhalation.
10
3. A therapeutic composition for treating pulmonary hypertension comprising a non-coding strand of the ET-1 gene, or fragment thereof, as represented in Figure 1; and, optionally, in combination with a suitable carrier.
15
4. A therapeutic composition according to Claim 3 wherein said non-coding strand of the ET-1 gene is a oligodeoxynucleotide, or a modified oligodeoxynucleotide, or a oligodeoxyribonucleotide, or a modified oligodeoxyribonucleotide.
20
5. A therapeutic composition according to Claim 3 or 4 wherein said non-coding strand of the ET-1 gene is between 10-100 nucleotides in length.
6. A therapeutic composition according to Claims 3 or 4 wherein said
25 non-coding strand of the ET-1 gene is greater than 100 nucleotides in length.
7. A therapeutic composition according to Claims 3-6 wherein said oligonucleotide has a G + C content of at least 50%.

8. A therapeutic composition according to Claims 3-7 wherein said oligonucleotide has a G + C content of between 50% and 60%.
- 5 9. A therapeutic composition according to Claims 3-8 wherein said oligonucleotide does not contain a consecutive run of more than four bases of any single kind.
- 10 10. A therapeutic composition according to Claims 3-9 wherein said oligonucleotide has a predicted melting temperature of at least 55⁰ C.
11. A therapeutic composition according to Claims 3-10 wherein said oligonucleotide has a predicted melting temperature of between 55⁰ - 80⁰C.
- 15 12. A therapeutic composition according to Claims 3- 11 wherein said antisense ET-1 molecule is of human origin and comprises at least one from;
- i) CAG CCC AAG TGC CCT TTA AC
 - ii) CTC AAA GCG ATC CTT CAG CC
 - 20 iii) AGC TCA GCG CCT AAG ACT GC
 - iv) TGG CAG AAG TAG ACA CAC TC
 - v) TGG TCT CCG ACC TGG TTT GT
 - vi) ATG TGC TCG GTT GTG GGT CA
- or an oligonucleotide sequence homologous therewith.
- 25 13 A therapeutic composition according to Claims 3-12 wherein said antisense ET-1 oligonucleotide is of rat origin and comprises at least one from;
- i) ACA GCA GAG AGA AGA TCA CG

- ii) TGC ACT TCC TTC TCA GCT CG
- iii) GGA TCG CTT AGA CCT AGA AG
- iv) CTT GAT GCT GTT GCT GAT GG
- v) AGT CAA TGT GCT CGG TTG TG
- 5 vi) ACT GTG TCT CTG CTC TCC GA

or an oligonucleotide sequence homologous therewith.

14. A therapeutic composition according to Claims 3- 11 wherein said non- coding strand of the ET-1 gene is adapted to hybridise to the
10 transcriptional promoter region of the ET-1 gene to inhibit transcription of said gene.

15. A therapeutic composition according to Claims 3- 11 wherein said non-coding strand of the ET-1 gene is adapted to hybridise to splice
15 junctions between exons and introns of prepromessenger RNA encoding the ET-1 protein.

16. A method for determining the efficacy of a therapeutic composition in the treatment of pulmonary hypertension comprising administering a
20 composition according to Claims 3-15 to a selected human or animal and observing the effects of said composition on pulmonary hypertension.

17. An inhaler comprising means for administering the therapeutic composition according to Claims 3-15, wherein said therapeutic
25 composition is administered via a pulse or spike during use of the inhaler.

18. An inhaler according to Claim 17 wherein the inhaler is disposable.

19. An inhaler according to Claim 17 wherein the inhaler is adapted to receive a cartridge containing antisense molecules according to Claims 3- 15.
20. An inhaler according to Claims 17 -19 wherein said inhaler comprises
5 a removable cartridge wherein said therapeutic composition is stored prior to use.
- 21 An inhaler according to Claims 17-20 wherein said inhaler comprises a rechargeable cartridge in which said therapeutic composition is stored prior
10 to use.
22. A method for administering a therapeutic composition according to Claims 3-15 comprising;
- 15 i) adding or providing said therapeutic composition in a suitable dilutant, carrier or excipient and in dosage form to, or in a reservoir of, an inhaler;
- ii) aerosolising said composition by suitable means;
- iii) detecting the air pressure within the inhaler by suitable sensing
20 device to determine a patient's respiratory cycle; and
- iv) delivering a prescribed dose of the therapeutic composition to the patient's lungs during a selected inhalation phase of the patient's respiratory cycle.
- 25 22. A kit for treating pulmonary hypertension comprising a therapeutic composition according to Claims 3-15, an inhaler according to Claims 17-21, an air pressure sensing device, and a valve containing pressurised cylinder means to regulate the dosage of said composition.

Figure 1

1/7

1 GATATCCTAT TAATACAGAG ATACAGAAG AAATACATTA AAAATAGTTT TATCAAATAC
 61 TTTCAGCAT TCAAGTGTAG CCTCAAAAGC AAGATAGGC CAGAGTGGT GGCTCACGCT
 121 GTAATCCACA GCACTGTGG AGCCCAAGGT AAGAGGATTG CTTGAGGCCA GGAATTCAAG
 181 ACCAGCCTAG GCAACATAGT GAGATCCCTA TCTCTACGAA AAAATTTTAA AACTTAGCTG
 241 GGCATGGTGC TTGAGCCTGT TGTCCAGCT ACTCAGAGAG TGAAGTAGGA GTGTCACTTG
 301 AGCCCAAGAG GTTGAGGCTG CAGTCAGCTA TAACTGCACC ACTGCACTCC AGCCTTGAG
 361 ACAGAGTGAG ACCCTGTCCC CAAAAAATTT AAATTGAGA AAAAAAATAA GGCAAGAACA
 421 GCCACAGCAA ACTTCTATTT GGGGAAAAAA AAAATTCCTC CTCTTTACAT CTCTCCCTTC
 481 CTCCCTTCC CTTCTGAGA GTGACTGTGG CCAAAAGGAG CATTTTCCCC CTGAGTCCCT
 541 CTGAGGGGTG GGGTGGGCT ATGAAGCTAT CCTTCATATT CACTCCCTTTG TCCAGCTCTT
 601 TTCACCTCTA GTTCTTCTCC CCGCATCTCT GTCTAGCAGT GCCTTAAGTG GAGAGGGGT
 661 GGGGGCATCA AGCTTGTAAA ACCTGTTTGT TGGGGTCTTC CTCTCCCCCT CATTTCTTGA
 721 TTCTTGGGAA AATGTCTTGC TGGAGGCTG CCTGGCGAGT GCCCTAGCTG CTTTCTGTGG
 781 GCTTGAATGG GCCTTCCCTC TGCCCCCTACA GAGGAAAAAG GGAAGTGTCTG CCAGAAGGAG
 841 AAATGAGAG ATGACACAGAG AAGGCAAGTG CCACCCCTCG CCCCTGACAC ACAAAAGAAA
 901 AGACACGGAA ATTCTCTCTC TCTCTTCTCT TCTCTATCT CTCTCTCTCT CCTCTCTCT
 961 CTCTCTCTCT CTCTCTCTCA CACACACACA CACACACACA CACACACACA CAGCGCGCG
 1021 CCGCGCGCGC AGGCACACGT CTTGCAAAAT CAGGATTCAA AGACACAGG GCACCATTA
 1081 ATTTGGCAGC GTGGGCTT CCAGTCTGA AATCCTGCAT TCTTCTTAC TATTTACTTT
 1141 CCCCAGCTC GAGAAAGGCC AGGTGTGGC GGATGGCTGG CCACGTTTGG TGTTTCCAAT
 1201 TCATATTAC GGGATGACAC AGACGGGCG TGGTAGTGC TGTGAGAGC GCTTGGCAG
 1261 TTTCAATTG CCCCCTCTT CCACCTGAAG GCTGGGCGTT GCTGGAACCT GCAAGGGCAG
 1321 CCTCAGCAAG GTGGGTGGC GTGGAGTGG GTGGAGAAAG GGACTCCAGC TGAAGTAGAA
 1381 CCCAGGCTGG ACCTGAGAAAT ATTGGGAGG GCATGGGCG TGGTTTCCG GTAAGGGCT
 1441 TGAGGACATG TTGCTCTGA CTGTGTGAG TGTGTGCTA AAGTTGCCAA AAGGTTAAAA
 1501 AAAAAAAGT AGGGGAGTC CCTGCCAAGA CATATTTC CAGGCCACTT TCTTCCGCG
 1561 GAGTGTGGG GGGGAGGCG TGCCTTGAAC CTGTGAATGT GACATCAGCT CTCTCTCTCT
 1621 CTCCCAAGT CGGCTTTGGA GAGGAGGTC AGGGCACCTT TGCCTGGCAC AGGCACGCTG
 1681 GCTTCCGCT CAGTGCCGCC TGTCTTCCG GAGCTGTGCG CTCCCTGGGC CCCGGGGCTA
 1741 GGCTGAGGTA AGCGACACAGC GGAGGCCAAG CGCGCCGCGA GAGGCTTGGG GGATAGGCTG

Figure 1-2

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1801 GAGGCATCTC TGGGTGTGG TGTGGTGTG GGTGTGGAG GGAGCTTCT TGCCTCTCTC
 1861 TCTCCCATCT CCACTCTTG CTTCAGTGG TCTTTAGAG GATGCATGTC ATTATGACC
 1921 TGTGCTGCC ACTGTCCCTG TCCCCCAGC TGTGACTTCG AGGAGGTCT GGGATCTGA
 1981 GTCTGTCCA ACCCAGGCT TGTCTGTGG GATAAAACT GTCTTTTGA TTTTAGAAG
 2041 AGGAGGAAA AAAGTTTCC CAGCATGTG GTTGTGCCAG TCTTGAAAT TCATCCGTGC
 2101 TTGAATCCA CCTCCATCC CCAGAAAAAC TGGAGTAAAA CAAAAAGAG AGATGACAA
 2161 AGTGTGTATT TGATGGCATC CCTGGGAG AGACTCTAAA TTTATCCCAT AGTCTTACT
 2221 GGGCCACTGT GAGCGCTTG GTGAGAAC AACAATAAT CTGGTGTCT AGTTGCTAA
 2281 CCTGAAAAAT GGGACTAGCG GAAAAAGCA ATGTGTCCA TGCACCTTT GCTTCTTTA
 2341 TTAAGCATG ATGTCACCTG TACAGTAAT GCCCTGTGT TACTTCAGGG GGGATTTCA
 2401 AGGTAGATA GACAGGAAAT TGTTTGAAA ATGTAACAC ATTATTAAT GTGAAGTATT
 2461 ATCTGATTCC TTGTTGCAAT GGCAITTTCT TCTCAGCAC ACCTTCTTG CATATTCAC
 2521 TAACCTGTGA CAAGAACACC TTTTGGCCCT AAATGAAGAC ACCCCCCCA AAAAAAGAGT
 2581 CCCAGAAAAAT ATGTCCCTGC TTGTGCGGA ATAAATAGAA TATCTGAGG TGCATTCTC
 2641 CTTCCTATGT TAGGCAACAT TCCCTGACCC TCCTCGGCC CCAAGCCAGG TTGCGTTTPT
 2701 TTCTGCCATT TAGAAGGTT TTCTTTTGT TCTTGTGTA ACATCAGCCC CTGTAGCTCT
 2761 TCATCTCCC CTGGTGTCT TCTCCGCCA TGTCTTAAAG TTGTTGGCAC CGACCAATCT
 2821 TAAGATTTAA GTTCTGTGTG AAAAAACACT TTGCTTTTCA ATCAGTTTAT CAGCTCTCTC
 2881 CGCAGGGGAA TGTGACACA CAAAAGAACT TATCGGGCT TCTCATCAGT GATAGGAAA
 2941 AGACTGGCAT GTGCTTAAC GAGCTCTGAT GTTATTTTA AGCTCCCTTT CTGCCAATC
 3001 CCTCACGAT CTTTCTCCGA TAGATGCAAA GAACCTCAGC AAAAAAGACC CGCAGGAAG
 3061 GGCTTGAAGA GAAAGTACG TTGATCTGCC AAAATAGTCT GACCCCACT AGTGGGCACT
 3121 GACGAGGAG AGCATTCCT TGTTGACTG AGACTAGAA CGGAGAGACA TAAAAAGAAA
 3181 ATGAAGCAG CACAATTTA AAAAAATTCC CCGCACACAA CAATACAATC TATTTAACT
 3241 GTGGCTCATA CTTTTCATAC CAATGATATG ACTTTTTC TGGAGTCCC TCTTCTGATT
 3301 CTGAACCTCC GGGGCTGCA GCTTGCAAG GGAAGCGGA CTCACGACT GCACGGGAG
 3361 GTTTAGCAAA GGTCTCTAAT GGGTATTTTC TTTTCTTAG CCTGCCCC GAATGTCTAG
 3421 ACGCGGGCG TCTGCTCTG AAGTTAGCAG TGATTTCTT TCGGGCTGG CTATCTCCG
 3481 GCTGCACGT GCCTGTGCT GACTAATAC ACAATAACAT TGTCTGGGGC TGAATAAAG
 3541 TCGGAGCTGT TTACCCCCAC TCTAATAGGG GTTCAATATA AAAAGCCGCC AGAGACTGT

Figure 1-3

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3601 CCAAGTCAGA CGCGCCCTCTG CATCTGCGCC AGCGGAACGG GTCCCTGCGCC TCCTGCAGTC
 3661 CCAGCTCTCC ACCGCGCCGCT GCGCCTGCAG ACGCTCCGCT CGCTGCGCTTC TCCTCTGGCA
 3721 GCGGCTGCCCT TTCTCTCCCG TTAAAGGGCA CTGGGCTGA AGGATCGCTT TGAGATCTGA
 3781 GGAACCCGCA GCGCTTTGAG GGACCTGAAG CTGTTTCTCT TCGTTTCTCT TTGGGTTGAG
 3841 TTGAACGGG AGGTTTTTGA TCCCTTTTCT TCAGAAATGA TTATTTGCTC ATGATTTTCT
 3901 CTCTGCTGTT TGTGGCTTGC CAAGGAGCTC CAGAAACAGG TAGGCACGCT CGTTGACTTG
 3961 TAAGTCTCGG AATTACAAGT TAGTGTGTTT TTAATCCACT TCATGCTTTT CTGCTTCTA
 4021 TTTTTCCTCCG TTCTTTTAT GACTGCAGCT TAGAGAGCAA GTGCTGAGA ATTAATGCTG
 4081 AACGTAAGT TAAGCTTCT AGGTAAAT GTAAATTTCC TCTACTGAAT ACAATTAGGT
 4141 GCAATTGACT ATACATGAC ATTAATAATA CTATCGTTT TATATTTATTT ATTCCATTAT
 4201 GTGTTTCCCTT GCGTTTAA AAATGAGAGG AGTATGACA TATACAATTT AGTCAATATG
 4261 ATGTTTGTAA TATATGTGT TATACAGGTA CACAGGCCAT ATAGGAAGTT AAATCTTAT
 4321 TAAACACTAT TTTAATAGTG TGTAAAGTG TAAATATTT AAGCATTTCA GCTTGAAGCC
 4381 AAGGAATTGT ATCCAGTCGT TCAGCAATG TATGTTCAAT AAAATCACTT GCAGAGCAAA
 4441 AGTCTGTTGA CTAATACCG CCTCCCCCGC CCCCCCAGG GCGGTTCTG
 4501 GGTGAAGCAG ATGTTTCTT TAAATTTTGT CATCATGAC TTTAGGTTTC TTTTGGCAGG
 4561 TTTTGGCAC CCAAAACAGT GTGAGCTCTC TTTTCAAGCTT TATTCACCTG TGCTGGGAGG
 4621 GGAGCTAGGA TAATCTTGG CTGCCGAGAG ATTTAGGCAG TCGGTGTGCA TCTGCCCGGG
 4681 TCCCCCCCCG TTTTAGGCTC AGTGCACTTT TTTTGTCTTT TCGTGACCTT GACTAAAGAG
 4741 AAAGATGTC AAGGAATGA AAATCCTGGA ATGTGTCTGA TCATTTGAAG TGTACAAAT
 4801 TGGCAGATA AGCTGCATGG CTAATTTGTT AGGAGGAAGA GGCAGGCAG TAGTGAGAA
 4861 GGGGAGGCA GTGATCCCA CACAAGCCTG ATGCCAGAGG ATTGGAATTT CAAAATCCCC
 4921 CCAGCCTACC TTCAGTCCCC TGACCCTGCTT CTCAGCCCCA CCTTAGGTCA CTGGTTCTA
 4981 TGAGTTTACC CTACTGAATT GAATATTGAA TAGTTAATTT CTCTCTCCAA TCATTTTCCC
 5041 CACCTAATTT TGAAGATAT ACATCATCTG GGGTACCCTG TGCCCTACAC AGCATGTGAA
 5101 GTGATGGGT ACCCCCTAAA GAGAGGCTCA TCCTGAATGG GGAAGTGGCC CCAAGCTAG
 5161 GAATAACTGT GATTTCTTGT CTTTATGTCAT GTGCCAATGT TAAGTAAAGT TCAGTGATA
 5221 GTGCTGTCTT ACCAAGTTCC TTGTAGAAGC CAGCCGGATT TTCAACAGGC AGCATTCAC
 5281 AGCATTTCCC TGAGCCTGCT TCAAGAGGGG TGGGGGAAGT CCCTTTTCAG GTGTTATCT
 5341 CCTCTGCATTT TGTGTAATCT CCCTGAAGGT GGATTAAGCCA AGGCATGAG GGGGAGGCA

Figure 1-4

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5401 AAGGTGAAC T CATGTTAAG AGGAAAAA ATAAGAGCC CTTTTTCTG TGTTCCTGC
 5461 TGATGGCAGG CTGTGTGCTT CATCTGCTT TATCTGCTT GCTAGCTCTG ACTCTACTGT
 5521 GATCCAGCAT GTCTCTCGGC GTTTGAGGAG ACATCCCCA CTGACCTGCT CTTTCTCTCC
 5581 CCAGCAGTCT TAGCGCTGA GCTCAGCGCG GTGGTGAGA ACGGCGGGA GAAACCCACT
 5641 CCCAGTCCAC CCTGGCGCT CCGCCGCTC AAGCGCTGCT CTTGCTGCTC CCTGATGAT
 5701 AAAGAGTGTG TCTACTTCTG CCACCTGGAC ATCATTTGGG TCAACACTCC CGAGTAAATC
 5761 TCTAGAGGCG ATGTAAACC TATTCATTCA TTAGCGCTGG CTCCACTGGA GCCCAGTTT
 5821 AGAGTTTCTT TTCTAGGGAC TCTGAAGGTA GTCCCTTCTAA CACCATCCAA GTGCTCAGT
 5881 GGGGACAGTT TCCCTCTATT CCTGAATAA ACGACAGCTT CGTTCTTAGC AACCAAGGG
 5941 AGGCTCTCTT GAGGCCCGCT AGCTCAGGCT ACTCATGATG GGACACAGC GAGGCCACTG
 6001 CACGTTTCAA ATGAGGAAC TTTCAAGTGA GGGCTCAGG GGGACACTCT CACAGTGGCA
 6061 TCTGATGGG GTTCGGGAAT AATTGCCGAG GTGAGATGTG GGTAGTGCA ACCGTGCTT
 6121 CTCATGGGAG GGTGGAGACT GAGAGGCAGA AGTGATGATA TAGAGGTTA GAATCACTTA
 6181 ATTTTAGTTA CAGAAAAACC TAGGCTCAAA GTGTTGAAGC CATTTGTGCA GGAGTGAATT
 6241 TGTAGCAGAG CTAGAACTGG AGCCCGGATTT TCCTTTGCTG CTATATTTTC CCTTAGAAA
 6301 TGCCCATTTT AGAACTGAAA TAGAATAACT GTCCATAGGC TTCTCTTTCA CCTACAGAGA
 6361 AGAAAAAGCAG ATTTCTCTCT TCTGCCCTGG ACACTAGTTC ATCATCTGTC GGAAGCAGTC
 6421 ATAACAAGC ACACATTTAC TATGCAATACA ATGTACCCTT ATGACCAAGG AGGACCAAAA
 6481 TCCAACAAT ATCAAAACCAC ACCAAAAAAC ACAAGGAGCC TAATAATTAC TAAAGTGATA
 6541 CTTCCAAAGG GAGGACTTTA TTTCTTAGAT GAGAAATGAAA ATGACACAT TGAATAATTAT
 6601 TGAGAGGCC TCTGGCTATG AGTCCTTCCA CAACCATATG GTACCAACCA CTGGCAGGAG
 6661 AAATGTGTGA ACATGTGCTT CCTCTCCCA ACCACTGGG CCGGTGGGT GACGGTGGCA
 6721 CTTTtagcag TATCCTCCGT GGTTTGAGTT GAAATTAAGT TTTAAAAATC CTGTGAGTCA
 6781 TGGTTTtGCA TTGAACCTC TTCCCACTGT GTACCCACAA ATAGTTAACT AAATAGACCA
 6841 TTAGAAAAGG AAGAAATAT AAAGCAGATG CCAAGCAGAG ATGTCTTAAT TTTTGACAAA
 6901 AAAGCAATGT TGTGTGTGTC AAGAAGAAAC TGAACCTTGT GAAGAGTTGA AATGAATTC
 6961 CACTGAATTA GAAAAACCTG TTTTCTCTCTG CCTGATGACA TACAGTCAGG GCCATTGATG
 7021 CACAGGTGTT CCTGGCTGTT GTTACACTTT ACCCTCTGAA ATGATGCTCC CAAGTCTAT
 7081 GTGATGAGCT CCTTGTGTGC CCAGTGAAT AGGTGTGTC ATGTGTCATT TTAAGAAGCTA
 7141 TTAATTACAC TAATATAGTT TCTTCTCTC TTTTGATTAAT AGGACCGTTG TTCCGTATGG

Figure 1-5

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7201 ACTTGAAGC CCTAGGTCCA AGAGAGCCTT GGAGATTTTA CTTCACACAA AGGCAACAGA
 7261 CCGTGAGAAAT AGATGCCAAT GTGCTAGCCA AAAAGACAAG AAGTGCTGGA ATTTTGGCA
 7321 AGCAGGAAAA GAACTCAGGT GAGCAGAAAC ACCTTTGCTT TTCAATCAGT TTAACAGCCCT
 7381 CCTGAACCTCC TTCCTATCAT GGTACTGCCCT TCCTGTTTGA GAGAGACTAA CAGAGACATTT
 7441 GAAAGTCAGG GTAAGCTGA ATATAACATT GCTGAATGCT TTTTCCCTGT GTATTTTAAAC
 7501 AGGCTGAAG ACATTATGGA GAAAGACTGG AATAATCATA AGAAAAGAAA AGACTGTTCC
 7561 AAGCTTGGGA AAAAGTGTAT TTATCAGCAG TTAGTGAGAG GAAGAAAAAT CAGAGAAAGT
 7621 TCAGAGGAAC ACCTAAGACA AACCAAGTAA GAGGGAAGGA AGAAAAATTA GGTAAAGAGT
 7681 TCACAGAAGAC AACTAGCCCC AGTCAGTGAT GCCAGCAGCC TGTTCCTCCA GCCCTTCTTA
 7741 CCGGGCAGG TGAAGACTT AGAAAAACAGT AGCAGAGGAG ATCTATGCAT CCTATAGATTT
 7801 AAAAGGAGCA AAGAATCCC TCTTAAATAT TTCCATGAAG CTCTGGAATG CAAAACCGATG
 7861 TCCTCTGTAC CTTTACACA TACCATTTGA TCTACAGGTA GATTTCCTCA CCAAAATATA
 7921 TCCAGAGATG CCTTGTGAT TGGGTATATAT ACAGCCTTTG CCTCTCTGAG TCAATGTATTT
 7981 TACCACCTTC CTGAGAAAT CGAAATCAT TTTGGGAGC GGACATTTAG AAAAAGAATC
 8041 AAAGTGTGAT GGATATATCAA ATTCTCAAT AAGTGCAGT TATTCAGATG GCCAAAGGAA
 8101 AAATAAAGTC ATTAGATAGG GTTGGTAGAA TTTAGAACAT GCTGTTTTTC AGGTTTATGG
 8161 TCTTTTTTTT TTTTTTTTTT TTTTTTTTAA TAGGGAATG TGTTTGGTGC AGAGCCAATG
 8221 TCATTCCAAA AAGCTCTCTC TTTTCCCTGT CAGTCATGTG CTGGACAGA GAAAGGATCT
 8281 GGATTAGGCA ACATCATAGA GTTGCTCTGA GCTGCTCTTT GGTGATAACC CTTCCAAATC
 8341 CTAACCTTTT TGAATTCAC AAGCTCAAG GAGGAAACCT ACTCTCTGAT GGCACAGATG
 8401 TTCTGCATTT TTCTATCATG GTCTATGAA ACTTCTCTTA GAAATCCAGT TGAGTTGTTT
 8461 TCTATGATTA AAGTGTCTG AGCTCAGGCC AGGCACTCAT GAACCTACTC TGAGTTGTTT
 8521 ACTACTGATT TGTGGGGCAG CCTCAGCTAT CGGTTTCTTC ACACCTGCTT ATGAGAGTAT
 8581 CCATATTTAT GGTCCGAGGC AGTAATGCTC CCCACGAGAT CAGTTTCTGA ACTAACCTGG
 8641 AATTTTTTAT GGGTTTTTAT TATGCCAACT ATTAATCAAA CATTAACAGTT CTTCCTCTG
 8701 TATTTCTCTT GTAAACATTT AGGCCCTGCAA AAAAAAATAA TCTTTTAAAA AATAATGCC
 8761 ATAAAGTATTT TGCCTGGGC CTACTGTATG CTTCCTTTTAT TTTTCTCTCT TTTCAACTAA
 8821 GTCAACCGTCA ATTATTTAAG ATGGCCATAA CTATTCAAAA CCTATGCTGA GTTCCTCAAG
 8881 GCAGGCTCGC ATAGTGATGA AGGTTGGGAT GGGGCTACGG AAGAACCAG AACAACTCTA
 8941 GTTTATTTAA AACCTGTATTT TACTGCCAC TTCCCTTAG ACTTGACCAT ATGACCCCTT

Figure 1-6

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9001 GCTCCCCATT CTAAGCATAG GGGCAGGCTT TATTTTACA ATGTAATAG ATGATATCAC
 9061 TTGAGGTTT ATCAAGAGT TGCGCGGGT GGTGAAGTT CACAACAGA TTCAGGTTT
 9121 GTTGTGCCA GATCTAATT TTACATGTTT CTTTGGCAA AGGTGATTT TTTTAAATA
 9181 ACATTTGTTT TCTCTATCT TGCTTTATTA GGTCCGAGAC CATGAGAAC AGCGTCAAT
 9241 CATCTTTCA TGATCCCAAG CTGAAGGCA AGCCCTCCAG AGAGCGTTAT GTGACCCACA
 9301 ACCGAGACA TTGGTGACAG ACCCTGGGG CCTGTCTGAA GCCATAGCCT CCACGAGAG
 9361 CCCTGTGGC GACTCTGCAC TCTCCACCCT GGCTGGGATC AGAGCAGAG CATCCTCTGC
 9421 TGCTTCTGA CTGCAAAAG ACCAGCGTCC TCGTTCAAAA CATCCAGA AAGTTAAG
 9481 AGTTCCCCA ACCATCTCA CTGGCTTCCA TCAGTGTAA CTGCTTGGT CTCTTCTTC
 9541 ATCTGGGAT GACAATGAC CTCACGACG AAACACACAG TCACATTCGA ATTGGGGTGG
 9601 CATCCTCCG AGAGAGAGAG AGGAAGAGA TTCACACACAG GGGTGGAGTT TCTGACGAAG
 9661 GTCCTAAGG AGTGTGTG TCTGACTCAG GCGCCTGGCA CATTCAGGG AGAACTCCA
 9721 AAGTCCACAC AAAGATTTTC TAAAGAAATGC ACAATTGAA AACACACTCA AAAGACAAAC
 9781 ATGCAAGTAA AGAAAAAAA AAGAAGACT TTTGTTTAAA TTTGTAAT GCAAACTGA
 9841 ATGAACCTGT TACTACCATA AATCAGGATA TGTTCATGA ATATGAGTCT ACCTCACCTA
 9901 TATTGCATC TGCGAGAAGT ATTTCCACA TTTAATTATT GCCTCCCAA ACTCTTCCA
 9961 CCCCTGCTGC CCTTCTCC ATCCCCATA CTAATCCTA GCCTCGTAGA AGTCTGTCCT
 10021 AATGTGTAG CAGTAGATAT AATATTTTCA TGGTAATCTA CTAGCTCTGA TCCATAAGAA
 10081 AAAAAAGATC ATTAATCAG GAGATTCCCT GTCCCTGATT TTTGAGACA CAATGTATA
 10141 GGGTGTGTTA TGAATATAT TGAAGAGTAA GTGTTGTTA CGCTTTAAG CAGTAATAAT
 10201 ATTTCCCTT ATATAACCG CTAATGAAG AGGTGGATT GAATTTGAT GTACTTATTT
 10261 TTTTATAGAT ATTATATTC AAACAATTTA TTCCTTATAT TTACCAATGTT AAATATCTGT
 10321 TTGGGCAGC CATATTGTC TATGTAATTT TAAATATGT ATTCTAAAT GAAATTGAGA
 10381 ACATGCTTGG TTTTGCCTGT CAAGGTAATG ACTTTAGAAA ATAAATATTT TTTTCCCTTAC
 10441 TGTACTGATT TGAATCATTT ACTGAAATTT GTAAGGAGTG GGCCAACGTG ATTTAAGTACC
 10501 ATAAAGGCAA ATAAATGTTT AAAGACGCTT TCATAGAAAA GTGACAATTA GAAGATATT
 10561 ACGGTCTAAG CTAATTATAT AAAGAATTTT ATCTGTATCT TAAATGTGA TTTTATACTG
 10621 CATGAGGTA AAAACACAAA ACAAAAAAGC AGCTTTAACA CCTCTGCTT CTCTTGGGTA
 10681 GCAGCCTCCT GCTTCTCCTT CACCTGAATA ATTCPCAGG GACTTCATCC ATTAACCTGG
 10741 CTCAGGCTAT TGGCAGGATT CACAGTTTAA GCTGATGGTG TGGTAGAGAGA TGCTTTATCC

Figure 1-7

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10801 ATATTATGCG ACTGAAGGAA GTAATGGCAA GACAACCCCC CAAAACATPAC CTAATTATAC
10861 AAAGTTATAT ACCAAAGTTG CTTTATAGAAA ATGGCCTGCT CAGAGCAAGT AGAGTTTCC
10921 AATGGCTTTT TAATTCTCTCA CATTAAGGAT GTTGTCTCTT AAGGAACATTT GAGTACCATTT
10981 GCTTCTTCGT GATAGCCCTAG GACTGCCGTG TGCCCATGGA GGTAGAGACA CCAGTACTG
11041 ATTCTAGGTC CTCTGCCACA AAGCACCACT TCCTCTCCAC TTTCCTTGG CTGGCCTTGT
11101 CAGCTCACTG GAGAGCACAG TATGCAATT GCAGTATTGC AAATGGTTC TACTAATCTGA
11161 ATTCTCTAAG AGCTGATTA GCCCTGAGA ATCTTCCCTG CCCTTCTCTA ATAGTGTCTG
11221 AAGGAATPCC TGGCATTTAA CAAATATTTAG CATGTAGTGA TCACGTGTCT CCTAACAGTG
11281 ACACATCAGA AGGATTTCAA ATAACAGTCT TCAGGCATGC GTAATCAATG TCCTGTGCAG
11341 AGTCTCCGTC CTCATTTGATC CTCATTTTTC TCTTTAAGGC ACAGTCCAAT GTCTTTGGG
11401 AATGTATTAT AAAGCTTACT TTATCCATAA ACTGTTTCTC AGTGCCTGAC TCTGAAGAAA
11461 ATTTGAAGT TTGCCCATG TTGACAAAGT GCTTGGTCTG AACTTGGCCA GTATTTAATC
11521 TTGAGCAAAC GATTCATTTT CCTTCTATCG TGAGTTTCTT CATCTATGAA ACAAGGAGT
11581 TGAGGGGAGT TTCTTTCTA CCTCTGAGAA AGAGTTTGAG ATTACATAAA GAAGTTGAAG
11641 TGGCATGAAA AAAAATTAAG ATCTGAGCTT AGAAGACATG GATCTAATAC ATTTAAGAGG
11701 AAGTCAGAAT CAGAGAAGCC ACTGAACAAA ACAGTCCAAA CGAGCATAG TAAGTCAGAT
11761 TGATGAGTTT TGGTTGGGTT TTTCATCAGT CAACCCCTG AGCCCCCTT TCCCATGCTT
11821 CCTGCTTCAG TATCCAGTAG GAAAAATGAA AGGATGATG TAGACACTCT AGGCATGAG
11881 GATTTGCAGT AAATTAAGTTG GGAGACTCAC AGAAATTTAA TATTTTCAA ACATGAAGAC
11941 GAAACATTC AATTATTTAC AGTCCACATC AGCTTGAAGG GTAAACTGAT GGGATGATCT
12001 GTCACATTTT TTGCTCTGTT TCCAGTAAAA GCATGTTTC TGAATAACCA CTTAGGACAG
12061 CTTTCTCTCT TTACACTGAT AGCCCAAGCA AGCTTTGATC TCAGAACTCC AGAAACAGA
12121 GAACPTTAGG TGAATGTGG TAACTTTTGC CAGGCGAGAG GGAACACCTA CTAATAGGTA
12181 CTTCAATTTG ACCACCAAG AGTTGGCATCT TTTTGTATGG ATCCACTGGC TTTGATACTG
12241 CCTGTACTCC CCCAAAACAC AGCTTGGGTA TTGGACTAAT CTAGAGCTCC CTGAGAGAA
12301 CTCTTGCTGA CATTAAGAAA GAGCAACAT TTGTCTTTCC AGGTGAAAAAT CCAAGGCCAA
12361 AAAGGGAGTG ACTCACCTAA GATCACAGAA GGAGCTGTAG CATCTCTGGA GCCTGAACAC
12421 TTAAGTTAAG CACGACTATT TCACGCAGAG GGCATGAATT C

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INTERNATIONAL SEARCH REPORT

Intel onal Application No

PCT/GB 98/02584

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/11 A61K31/70 A61M15/00 A61M11/00 //B65D83/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07H A61K A61M C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>SAKAI S ET AL: "Pulmonary hypertension caused by congestive heart failure is ameliorated by long-term application of an endothelin receptor antagonist : Increased expression of endothelin -1 messenger ribonucleic acid and endothelin -1-like immunoreactivity in the lung in congestive heart failure in rats."</p> <p>JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, (1996 NOV 15) 28 (6) 1580-8., XP002092833</p> <p>see the whole document</p> <p>---</p> <p>-/--</p>	<p>1,3-11, 16</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

10 February 1999

Date of mailing of the international search report

24/02/1999

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Andres, S

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/02584

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>WO 96 40162 A (UNIV EAST CAROLINA ;NYCE JONATHAN W (US); METZGER W JAMES (US)) 19 December 1996 see page 7, line 2 see page 8, line 23 - page 10, line 11 see page 11, line 17 - line 27 see page 38, line 20 - page 39, line 35 see claims</p> <p style="text-align: center;">----</p>	1, 3-11, 16
X	<p>GB 2 283 179 A (HIGENBOTTAM TIMOTHY WILLIAM) 3 May 1995 see the whole document</p> <p style="text-align: center;">----</p>	17-21
X	<p>WO 94 27664 A (KEANEY NIALL) 8 December 1994 see page 5, line 8 - page 7, line 18 see claims</p> <p style="text-align: center;">----</p>	17-21
A	<p>BUTT A.Y. ET AL: "Pathophysiological basis of the treatment of pulmonary hypertension." EUROPEAN RESPIRATORY REVIEW, (1995) 5/29 (248-251)., XP002092834 see page 249, right-hand column, paragraph 2 - page 250, paragraph 1</p> <p style="text-align: center;">----</p>	1
A	<p>STELZNER, T. ET AL.: "Increased lung endothelin-1 production in rats with idiopathic pulmonary hypertension" AMER. J. PHYSIOL., vol. 262, May 1992, pages L614-L620, XP002092835 see the whole document</p> <p style="text-align: center;">----</p>	1
P, X	<p>GB 2 320 900 A (UNIV SHEFFIELD) 8 July 1998 see abstract see page 3, line 18 - page 5, line 5 see claims</p> <p style="text-align: center;">-----</p>	17-21

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 98/ 02584

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-2, 16 and 22
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-15,22

Antisense oligonucleotides against endothelin-1 for the treatment of pulmonary hypertension.

2. Claims: 16

A method of determining the efficiency of a therapeutic composition in the treatment of pulmonary hypertension.

3. Claims: 17-21

An inhaler.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-15,22

Antisense oligonucleotides against endothelin-1 for the treatment of pulmonary hypertension.

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An inhaler.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/GB 98/02584

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